Mentype® **DIPplex** PCR Amplification Kit

Product description

Human identification is commonly based on the analysis of short tandem repeats (STRs) or single nucleotide polymorphisms (SNPs) depending on the demands of an examination or on the sample quality. While being able to merge the advantages of both STRs and SNPs, biallellic Deletion/Insertion Polymorphisms (DIPs) have yet substantial capability regarding identification in forensics or paternity analysis.
The Mentype® **DIPplex** PCR Amplification Kit was developed for multiplex amplification of 30 DIPs and Amelogenin. The primers are fluorescence-labelled with **6-FAM**, **BTG**, **BTY** or **BTR**.

In order to resort genotypes for easy interpretation of the results, it is recommended to use the special freeware **DIPSorter**.

DIP loci with short amplicon length have substantial benefits to analyse degraded samples with respect to forensic applications. Therefore, the maximal amplicon length within the Mentype® **DIPplex** was restricted to ~150 bp. A further advantage of DIPs is the absence of stutter peaks, which makes them suitable for mixed stain analyses. Furthermore, the 30 DIPs are distributed over 19 autosomes that are at least 10 Mbp away from any commercially available STR and SNP marker (see Tab. 1). Hence, in combination with standard markers, the new DIP markers improve the discrimination power and provide a potential supplementation for paternity analyses.

The detection limit of Mentype® **DIPplex** is about **100 pg genomic DNA**. However, it is recommended to use **0.2-0.5 ng DNA**.

The test kit was validated and evaluated using the GeneAmp® 9700 thermal cycler, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100/3130 Genetic Analyzer.

Table 1. Discrimination power of DIPs, STRs and SNPs

	Loci	**CPE/Trio ***	**CPI ****	**Population**
Mentype® DIPplex	30 DIPs	0.9980	2.83×10^{-13}	German
AmpFISTR Minifiler	8 STRs	0.99976	8.21×10^{-11}	US Caucasian
AmpFISTR SEfiler	11 STRs	0.999998	7.46×10^{-14}	US Caucasian
Powerplex 16	15 STRs	0.9999994	5.46×10^{-18}	US Caucasian
Sanchez et al. 2006	52 SNPs	0.9998	5.00×10^{-21}	European

*combined probability of paternity exclusion, **combined probability of identity

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1. Description of the Mentype® DIPplex

Table 2. Locus-specific information of Mentype® DIPplex

DIP Locus	Chromosomal Localisation	GenBank® Accession/ SNP ID	Motive (+DIP)	Reference Allele
DIPplex_Blue				
AM X	Xp22.1-22.3	M55418		X
AM Y	Yp11.2	M55419		Y
HLD77	7q31.1	rs1611048	TAAG	+DIP
HLD45	2q31.1	rs2307959	CACG	-DIP
HLD131	7q36.2	rs1611001	TGGGCTTATT	+DIP
HLD70	6q16.1	rs2307652	AGCA	-DIP
HLD6	16q13	rs1610905	GCAGGACTGGGCACC	-DIP
HLD111	17p11.2	rs1305047	CACA	-DIP
HLD58	5q14.1	rs1610937	AGGA	+DIP
HLD56	4q25	rs2308292	TAAGT	+DIP
DIPplex_Green				
HLD118	20p11.1	rs16438	CCCCA	-DIP
HLD92	11q22.2	rs17174476	GTTT	-DIP
HLD93	12q22	rs2307570	ACTTT	-DIP
HLD99	14q23.1	rs2308163	TGAT	-DIP
HLD88	9q22.32	rs8190570	CCACAAAGA	+DIP
HLD101	15q26.1	rs2307433	GTAG	-DIP
HLD67	5q33.2	rs1305056	CTACTGAC	-DIP
DIPplex_Yellow				
HLD83	8p22	rs2308072	AAGG	-DIP
HLD114	17p13.3	rs2307581	TCCTATTCTACTCTGAAT	-DIP
HLD48	2q11.2	rs28369942	GACTT	-DIP
HLD124	22q12.3	rs6481	GTGGA	-DIP
HLD122	21q22.11	rs8178524	GAAGTCTGAGG	-DIP
HLD125	22q11.23	rs16388	ATTGCC	-DIP
HLD64	5q12.3	rs1610935	GACAAA	+DIP
HLD81	7q21.3	rs17879936	GTAAGCATTGT	-DIP
DIPplex_Red				
HLD136	22q13.1	rs16363	TGTTT	-DIP
HLD133	3p22.1	rs2067235	CAACCTGGATT	-DIP
HLD97	13q12.3	rs17238892	AGAGAAAGCTGAAG	-DIP
HLD40	1p32.3	rs2307956	GGGACAGGTGGCCACTAGGAGA	+DIP
HLD128	1q31.3	rs2307924	ATTAAATA	-DIP
HLD39	1p22.1	rs17878444	CCTAAACAAAAATGGGAT	-DIP
HLD84	8q24.12	rs3081400	CTTTC	-DIP

Abbreviations: HLD = Human Locus DIP, -DIP = Deletion, +DIP = Insertion

Table 2 shows the DIP loci of the Mentype® **DIPplex**, their chromosomal localisation, GenBank®/SNP ID number, motive and respective reference allele.

Content

Mentype® **DIPplex** PCR Amplification Kit (100 Reactions)

Nuclease-free water	3.0 mL
Reaction mix **A**	500 µL
Primer mix	500 µL
DNA polymerase	60 µL
Control DNA XY5	10 µL
DNA Size Standard 550 (BTO)	50 µL
Allelic ladder	25 µL

Ordering information

Mentype® **DIPplex**	25 reactions	Cat. No.	45-31110-0025
Mentype® **DIPplex**	100 reactions	Cat. No.	45-31110-0100
Mentype® **DIPplex**	400 reactions	Cat. No.	45-31110-0400

Storage

Store all components at -20°C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents are needed in order to use the Biotype® PCR Amplification Kit:

Reagent	**Supplier**	**Order Number**
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards BT5 single-capillary instruments (5x25 µL)	Biotype Diagnostic GmbH	00-10411-0025
Matrix Standards BT5 multi-capillary instruments (25 µL)	Biotype Diagnostic GmbH	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (50 µL)	Biotype Diagnostic GmbH	00-10421-0050

Warning and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Primer mix, reaction mix and allelic ladder	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Trademarks and patents

Mentype® is a registered trademark of Biotype Diagnostic GmbH.
ABI PRISM®, GeneScan®, Genotyper®, GeneMapper™ and Applied Biosystems are registered trademarks of Applied Biosystems Inc. or its subsidiaries in the U.S. and certain other countries.
6-FAM, POP-4 and Hi-Di are trademarks of Applied Biosystems Inc.
GeneAmp® is a registered trademark of Roche Molecular Systems.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).
GenBank® is a trademark of National Institute of Health.

Protocols for PCR amplification, electrophoresis and analysis

2. PCR amplification

2.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µL reaction volume, including a sample volume of 1.0 µL (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate the pipetting error.

Component	Volume
Nuclease-free water	13.4 µL
Reaction mix **A***	5.0 µL
Primer mix	5.0 µL
Multi Taq2 DNA Polymerase (hot start, 2.5 U/µL)	0.6 µL
Volume of master mix	24.0 µL

* contains Mg^{2+}, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. A volume of up to 5 µL may be necessary for DNA trace templates. DNA volumes of more than 5 µL are not recommended, because potential PCR inhibitors may interfere with the process. Fill up the final reaction volume to 25 µL with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e.g. 0.1x TE buffer.

The primer mixes are adjusted for balanced peak heights at **30 PCR cycles** and **0.2 ng Control DNA XY5** in a reaction volume of 25 µL. If more DNA template is introduced, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

Positive control

For the positive amplification control, dilute the Control DNA XY5 to 0.2 ng in the appropriate volume. Instead of the template DNA, pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

Negative control

For the negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube which contains the PCR master mix.

Template DNA

Sometimes, the measured value of the DNA concentration varies depending on the quantification method used, so that it may be necessary to adjust the optimal DNA amount.

2.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Multi Taq2 DNA Polymerase and to prevent the formation of non-specific amplification products.

The number of cycles depends on the amount of DNA. 30 cycles are recommended for all samples. For critical stains (< 100 pg DNA), it is recommended to increase the number of PCR cycles from 30 to 32.

Standard method

Recommended for all DNA samples

Temperature	Time	
94°C	4 min (hot start for activation of the Multi Taq2 DNA Polymerase)	
94°C	30 s	
61°C	120 s	**30 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

Optional

Recommended for stains with small amounts of DNA

Temperature	Time	
94°C	4 min (hot start for activation of the Multi Taq2 DNA Polymerase)	
94°C	30 s	
61°C	120 s	**32 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

Too small amounts of DNA may result in allelic dropouts and imbalances of the peaks. Furthermore, unspecific amplification products could appear. With increasing numbers of cycles, there is the risk of cross contamination caused by minimal amounts of impurities.

3. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan® or GeneMapper™ ID software, refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan® software is described below.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	47 cm / 50 µm (green)
Polymer	310 Genetic Analyzer POP-4
Buffer	10x Genetic Analyzer Buffer with EDTA

3.1 Matrix generation

Prior to conducting DNA fragment size analysis with the filter set G5, a matrix with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO must be generated.

Colour	**Matrix standard**
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR
Orange (O)	BTO

Five electrophoresis runs shall be conducted, one for each fluorescent label, 6-FAM, BTG, BTY, BTR, and BTO, under the same conditions as for the samples and allelic ladders of the Biotype® test kit to generate suitable matrix files.

Matrix sample	**Component**	**Volume**
Matrix sample 1	Hi-Di™ Formamide	12.0 µL
	Matrix standard **6-FAM**	1.0 µL
Matrix sample 2	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTG**	1.0 µL
Matrix sample 3	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTY**	1.0 µL
Matrix sample 4	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTR**	1.0 µL
Matrix sample 5	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BT0**	1.0 µL

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

- Create a **Sample Sheet** and enter a sample designation

Injection list for matrix generation

Parameter	**Set up**
Module File	GS STR POP-4 (1 mL) **G5**
Matrix File	**NONE**
Size Standard*	**NONE**
Injection [s]	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]	24

* Prepare matrix standards always **without DNA Size Standard (BTO)**

Analysis of the matrix samples

- Run the GeneScan® software
- **File** → **New** → **Project** (open folder of current run) → **Add Sample Files**
- Select a matrix sample in the **Sample File** column
- **Sample** → **Raw Data**
- Check the matrix samples regarding a flat baseline. As shown the figure below, there should be at least five peaks with peak heights about 1000-4000 (Y-axis) for each matrix sample (optimal range: 2000-4000)

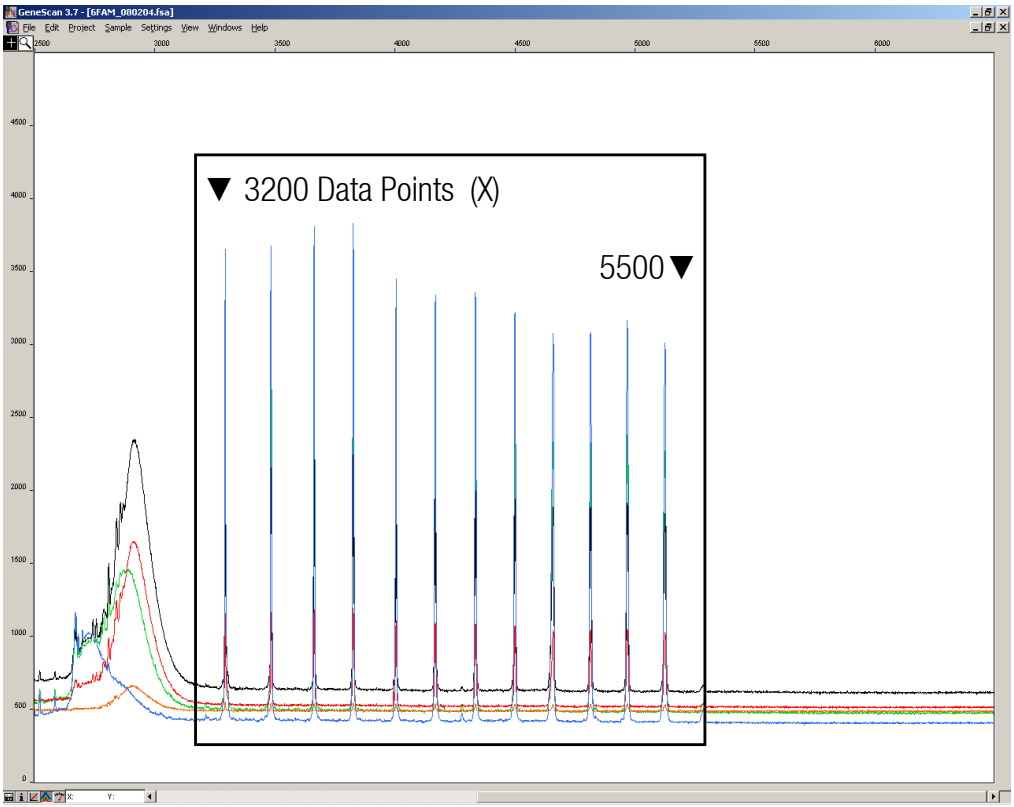

Fig. 1 Electropherogram with raw data of the matrix standard 6-FAM

- Select an analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

Generation of a new matrix

- **File → New → Matrix**

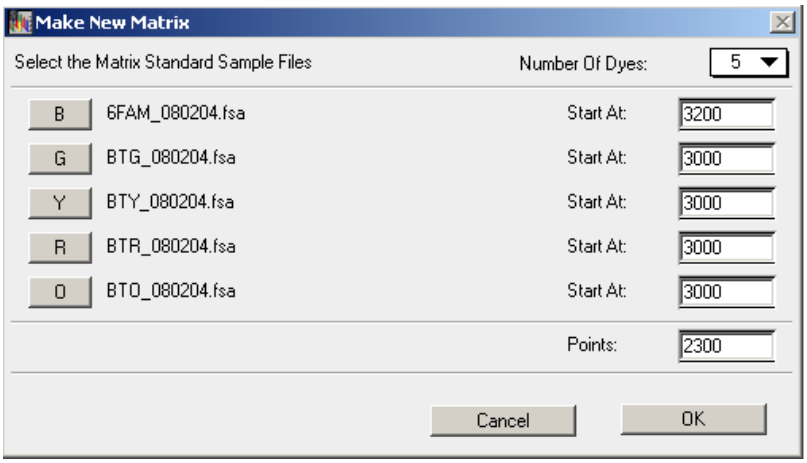

Fig. 2 Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a **Start At** value, e.g. 3200
- Enter the calculated difference under **Points**, e.g. 2300
- Click on **OK** to calculate the new matrix

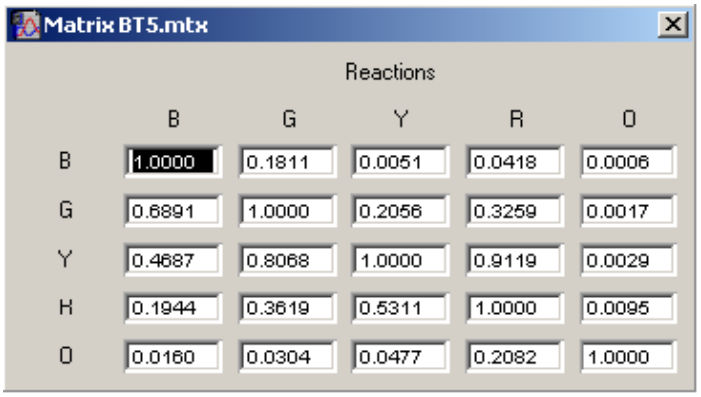

Fig. 3 New matrix BT5

- Save the matrix in the matrix folder: **File → Save as**, e.g. Matrix BT5

Matrix check

Check the new matrix with current samples.
- **File → New → Project** (open folder of the respective run) → **Add Sample Files**
- Select sample(s) in the **Sample File** column
- **Sample → Install New Matrix** (open matrix folder and select new matrix)
- Re-analyse your samples

There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

3.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Signal intensities

Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

3.3 Setting up the GeneScan® software

- Create a **Sample Sheet** and enter a sample designation

Injection list

Parameter	Set up
Module File	GS STR POP-4 (1 mL) **G5**
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-200bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**20**

* Deviating from the standard settings, the injection time may range between 1 and 10 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 10 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **DIPplex** was modified in order to be able to analyse fragments with lengths of up to **200 bp**.

3.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 6000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper™ ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

4. Electrophoresis using the ABI PRISM® 3100-Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, application of the ABI PRISM® 3100 Data Collection software version 1.01 or 1.1 and the GeneScan® software, refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*.

The system with 4 capillaries is named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	3100 Capillary Array, 36 cm
Polymer	3100 POP-4 Polymer
Buffer	10x Genetic Analyzer Buffer with EDTA

4.1 Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolour systems with the ABI PRISM® 3100-Avant/3100 Genetic Analyzer and shall be done prior to conducting fragment length analysis. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3100-Avant

Component	**Volume**
Hi-Di™ Formamide	60.0 µL
Matrix standard **BT5**	5.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3100

Component	**Volume**
Hi-Di™ Formamide	204.0 µL
Matrix standard **BT5**	17.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing a spectral calibration run

First of all, the parameter file for **DyeSetG5** must be modified once to achieve successful calibration with the Data Collection software version 1.0.1 or 1.1.

Spectral parameter

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select **MtxStd{Genescan_SetG5}** to open the PAR-file
- Change **Condition Bounds Range** to [1.0; 20.0]. If calibration was not successful, also change **Sensitivity** to 0.1 and **Quality** to 0.8 in a second step
- Select **File** → **Save As** to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_BT5}.par

Always use this parameter file for spectral calibration runs using Biotype® matrix standards **BT5**.

Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **Spectral Calibration**
- Select **96-Well** as plate type and click on **Finish**

Plate editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Type name for the matrix samples
Dye Set	G5
Spectral Run Module	*Default* (e.g. Spect36_POP4)
Spectral Parameters	MtxStd{GeneScan_SetG5_BT5}.par (parameters created before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information of the selected samples and confirm with **OK**
- Link your reaction plate on the autosampler tray with the created plate ID and start run
- On completion of the run check in the **Spectral Calibration Result** dialog box if all capillaries have successfully passed calibration (label **A**). If individual capillaries are labelled **X**, refer to *ABI PRISM® Genetic Analyzer User's Manual*.
- Click on **OK** to confirm completion of the run

Matrix check

- Select **Tools → Display Spectral Calibration → Dye Set → G5** to review the spectral calibration profile for each capillary
- The quality value (**Q value**) must be greater than 0.95 and the condition number (**C value**) must be between 1 and 20. Both values must be within the previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, try to change the **Sensitivity** and **Quality** values in the parameter file as described above
- If all capillaries have passed the calibration, the last calibration file for **Dye Set G5** must be activated manually under **Tools → Set Active Spectral Calibration**. Rename the calibration file under **Set Matrix Name** (e.g. BT5_Date of calibration)

4.2 Sample preparation

Component	**Volume**
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

4.3 Setting up the GeneScan® software

Edit the default run module in **Dye Set G5** once for the first run.
- Select **Module Editor** to open the dialog box
- Select the appropriate **Run Module** as template from the **GeneScan** table
- Modify the **Injection Voltage** to 3 kV and the **Injection Time** to 10 s

Run Module 3kV_10s_260bp

Parameter	**Set up**
Run Temperature [°C]	*Default*
Cap Fill Volume	*Default*
Maximum Current [A]	*Default*
Current Tolerance [A]	*Default*
Run Current [A]	*Default*
Voltage Tolerance [kV]	*Default*
Pre Run Voltage [kV]	*Default*
Pre Run Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Run Voltage [kV]	*Default*
Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Time [min]**	**15**

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions the Run Time for Mentype® **DIPplex** was modified in order to be able to analyse fragments with lengths of up to **200 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_260bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click on **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **GeneScan**
- Select **96-Well** as plate type and click on **Finish**

Plate Editor

Parameter	Set up
Sample Name	Type name for the samples
Dyes	O
Colour Info	Ladder or sample
Project Name	e.g. 3100_Project1
Dye Set	G5
Run Module*	3kV_10s_260bp
Analysis Module 1	DefaultAnalysis.gsp

* parameter see above

- Complete the table in the **Plate Editor** and click on **OK**
- Click into the column header to select the entire column and select **Edit → Fill Down** to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as **Color Data** in **Array View** of the 3100 Data Collection software or as **Analyzed Sample Files** under D:/AppliedBio/3100/DataExtractor/ExtractRuns

4.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2200 Stop: 6000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper™ ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

5. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper™ ID software, refer to the *ABI PRISM ® 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130, and the system with 16 capillaries is named ABI 3130xl.

The virtual **filter set Any5Dye** shall be used for the combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	36 cm Capillary Array for 3130/3130xl
Polymer	POP-4 Polymer for 3130
Buffer	10x Genetic Analyzer Buffer with EDTA

5.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

Component	**Volume**
Hi-Di™ Formamide	60.0 µL
Matrix standard **BT5**	5.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3130xl

Component	**Volume**
Hi-Di™ Formamide	204.0 µL
Matrix standard **BT5**	17.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing a spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Instrument Protocol for spectral calibration

Protocol Editor	**Set up**
Name	*User* (e.g. Spectral36_POP4_BT5)
Type	SPECTRAL
Dye Set	Any5Dye
Polymer*	*User* (e.g. POP4)
Array Length*	*User* (e.g. 36cm)
Chemistry	Matrix Standard
Run Module*	*Default* (e.g. Spect36_POP4_1)

* Depends on the type of polymer and length of capillary used

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection software, click on **New** to open the **New Plate Dialog** box

Plate Editor for spectral calibration (I)

New Plate Dialog	**Set up**
Name	e.g. Spectral_BT5_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_BT5 (setting described before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information to all selected samples, and click on **OK**
- In the **Run Scheduler** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run

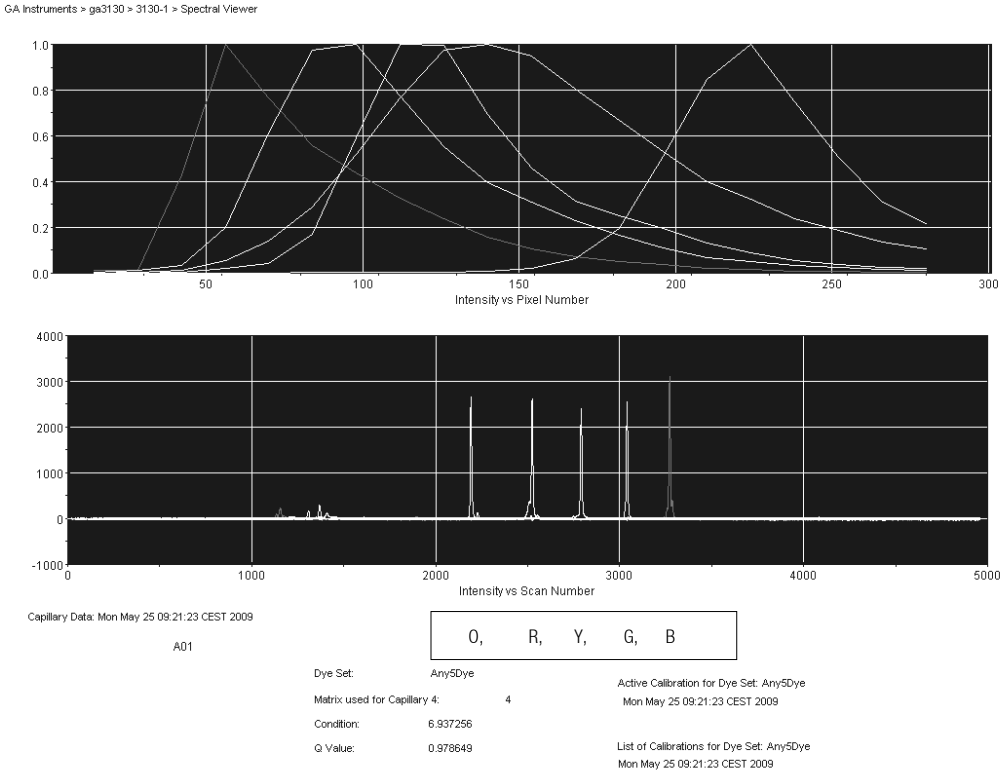
GA Instruments > ga3130 > 3130-1 > Spectral Viewer
Intensity vs Pixel Number
Intensity vs Scan Number
Capillary Data: Mon May 25 09:21:23 CEST 2009
A01
O, R, Y, G, B
Dye Set: Any5Dye
Matrix used for Capillary 4: 4
Condition: 6.937256
Q Value: 0.978649
Active Calibration for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009
List of Calibrations for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009

Fig. 4 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20.
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, use the optimised values and repeat the calibration run
- If all capillaries have passed the test, the last calibration file for the Dye Set **Any5Dye** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. BT5_Date of calibration) using the respective button

5.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

5.3 Setting up the GeneMapper™ ID software

Edit the run module as follows for the first run:

- In the **Module Manager** of the Data Collection software click on **New** to open the **Run Module Editor** dialog box

Run Module 3kV_10s_260bp

Parameter	**Set up**
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Voltage Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Voltage [kV]	*Default*
Run Time [s]**	**900**

* Apart from standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If samples with very high signal intensities are to be recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 20 s may be necessary.

** Depending on the analysis conditions the run time for Mentype® **DIPplex** was modified in order to be able to analyse fragments with lengths of up to **200 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_260bp) and confirm with **OK**
- To exit the **Run Module Editor** click on **Close**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software, click on **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box

Instrument Protocol

Protocol Editor	**Set up**
Name	Run36_POP4_BT5_15min
Type	REGULAR
Run Module*	3kV_10s_260bp
Dye Set	Any5Dye

* parameter see above

- Click on **OK** to exit the **Protocol Editor**

Prior to each run, it is necessary to create a plate definition as follows:

- In the **Plate Manager** of the Data Collection software click on **New** to open the **New Plate Dialog** box

GeneMapper™ Plate Editor (I)

New Plate Dialog	**Set up**
Name	e.g. Plate_BT5_Date
Application	Select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

GeneMapper™ Plate Editor (II)

Parameter	**Set up**
Sample Name	Enter name for the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or allelic ladder
Size Standard	e.g. SST-BTO_60-260bp
Panel	e.g. DIPplex_Panels_v0
Analysis Method	e.g. DIPplex_HID_3130_200rfu
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_BT5_15min (setting described before)

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler,** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

5.4 Analysis parameter / analysis method DIPplex

The recommended settings in the worksheet **Peak Detector** are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Partial Range Start Pt: 2200; Stop Pt: 6000 Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts Slope Thresholds: 0.0

Recommend settings in the worksheet **Allele** are:

Amelogenin Cutoff**	0.1

Recommend settings in the worksheet **Peak Quality** are:

Heterozygote balance	Min peak height ratio: 0.1
Allele number	Max expected alleles: 20

* The peak amplitude threshold (Cutoff value) corresponds to the minimum peak height that will be detected from the GeneMapper™ ID software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times higher then the background noise of the baseline.

** All DIPs will be examined by GeneMapper™ ID software like Amelogenin.

6. Analysis

For general instructions on automatic sample analysis, refer to the *GeneScan®* or *GeneMapper™ ID Software User's Manual.*

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some DIP loci, determining the size should be based on evenly Mentype® **DIPplex**. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550 bp**.

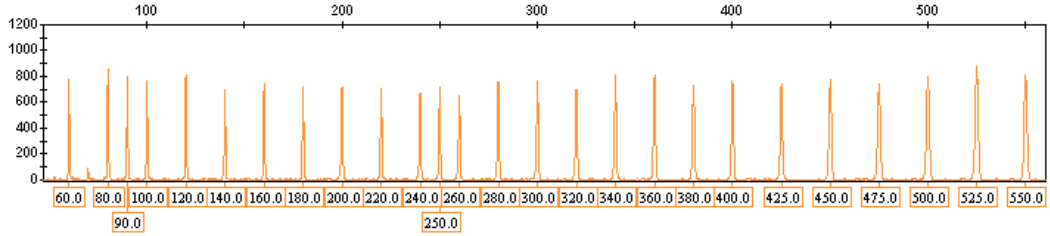

Fig. 5 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Mentype® **DIPplex**: The basic template files for the DNA Size Standard 550 (BTO) was adjusted to 260 bp within the GeneMapper™ ID software. An own created new template could be saved as e.g. SST-BTO_60-260bp and used for further analyses.

6.1 Biotype® template files

Allele allocation should be carried out with suitable analysis software, e.g. the GeneMapper™ ID or Genotyper® software in combination with the Mentype® **DIPplex** template files from Biotype. Template files are available from our homepage or as CD-ROM on request.

In order to resort genotypes for easy interpretation of the results, we offer the special freeware **DIPSorter**. For application of **DIPSorter**, please use the recommended **Table Settings** for displaying and exporting the genotype table from GeneMapper™ ID (Table for 20 Alleles) or Genotyper® software (Vertical Table for 20 Alleles).

Sample Name	Marker	Dye	Allele 1	Allele 2	…	Allele 20	…

Export Table from GeneMapper™ ID software

File Name	Sample Info	Category	Peak 1	Peak 2	…	Peak 20

Export Table from Genotyper® software

Recommended Biotype® templates for GeneMapper™ ID software are:

Panels	DIPplex_Panels_v0	or higher versions
BinSets	DIPplex_Bins_v0	or higher versions
Size Standard	SST-BTO_60-260bp	
Analysis Method	DIPplex_HID_310_50rfu	
	DIPplex_HID_310_200rfu	
	DIPplex_HID_3130_50rfu	
	DIPplex_HID_3130_200rfu	
Plot Settings	Plots_5dyes	
Table Settings	Table for 20 Alleles	

Recommended Biotype® template files for Genotyper® software are:

DIPplex_v0	or higher versions

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

6.2 Controls

The Control DNA XY5 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 3. Allele determinations of Mentype® DIPplex

Locus	Control-DNA XY5	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
AM	X/Y	X	X	X/Y	X/Y
HLD77	+	-/+	-	+	+
HLD45	-	-	-/+	+	-/+
HLD131	-/+	-/+	-	-/+	+
HLD70	-	-/+	+	-/+	-
HLD6	-/+	+	+	-/+	-/+
HLD111	-	+	-/+	-/+	-/+
HLD58	-/+	-	+	+	+
HLD56	-	-	-/+	-	-/+
HLD118	-/+	-	-	-	-/+
HLD92	+	+	-/+	+	-
HLD93	-/+	+	-	-	-/+
HLD99	-/+	+	-/+	+	-/+
HLD88	-/+	-	-	-/+	+
HLD101	-/+	-/+	-/+	-/+	-/+
HLD67	+	-/+	+	+	+
HLD83	-/+	-	-/+	-	-
HLD114	-	-	-	+	-/+
HLD48	-/+	+	+	-/+	+
HLD124	-/+	-	-/+	+	+
HLD122	-	-	-/+	-	-/+
HLD125	-	-	-/+	+	-
HLD64	-/+	-	+	-/+	-/+
HLD81	-/+	-/+	-	-/+	+
HLD136	-/+	+	-/+	+	-/+
HLD133	-/+	-	+	+	-/+
HLD97	-/+	-	-/+	-/+	+
HLD40	-/+	+	-	-/+	+
HLD128	-	-/+	-/+	-	-/+
HLD39	-/+	-/+	-	+	-
HLD84	-	+	-	-/+	-

The reference DNA K-562 is available from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines), DNA 9947A, 9948 and 3657 are available from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) that is up to standard of Szibor et al. (2003).

6.3 Lengths of fragments and alleles

Table 4 to **table 5** show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 310/3130 Genetic Analyzer with POP-4 polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

Horizontal: 70-205 bp
Vertical: Depending on signal intensity

Figure 6

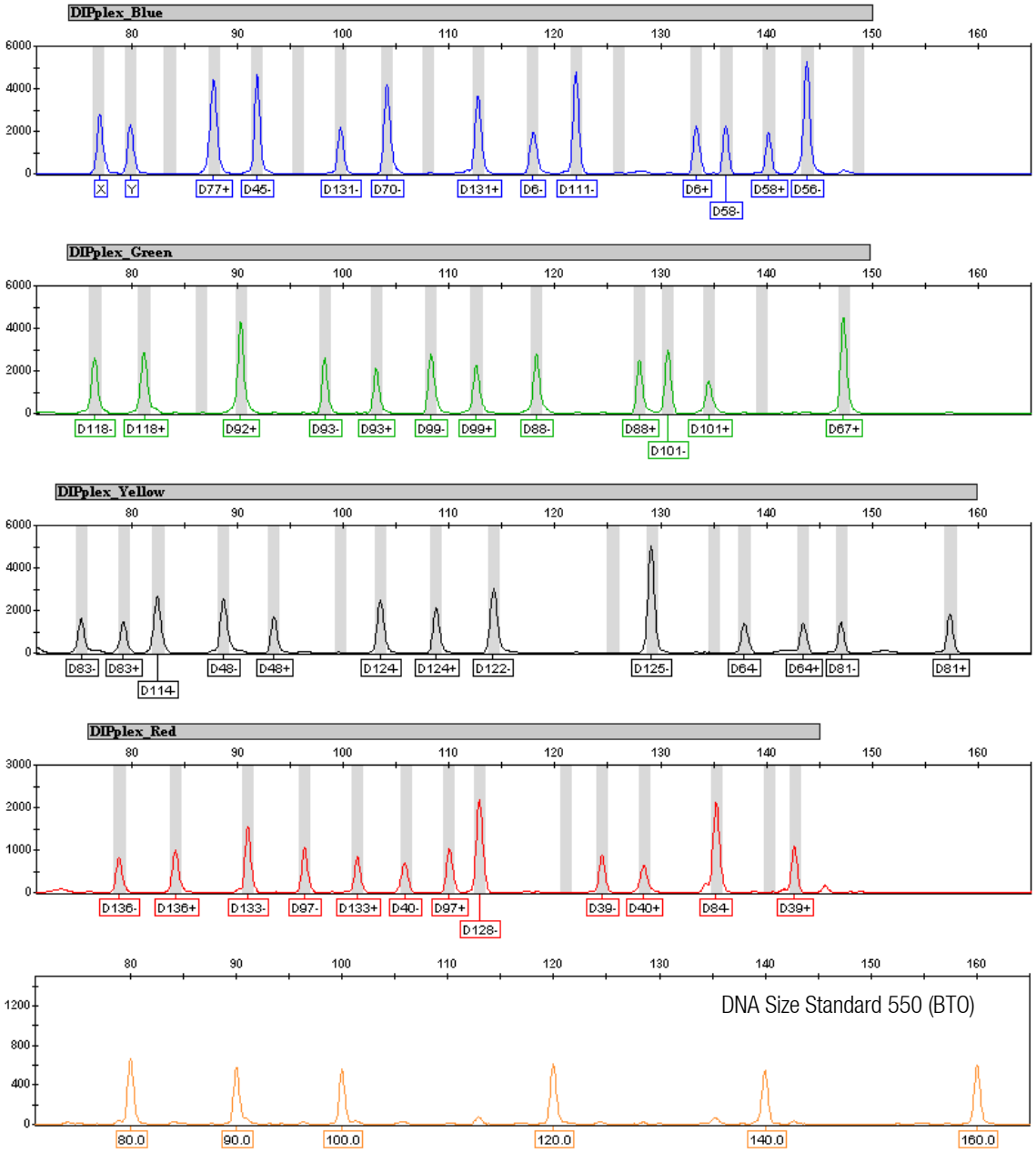

Fig. 6 Electropherogram of the Mentype® **DIPplex** using 200 pg Control DNA XY5. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper™ ID Software and the Mentype® **DIPplex** template file.

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Figure 7

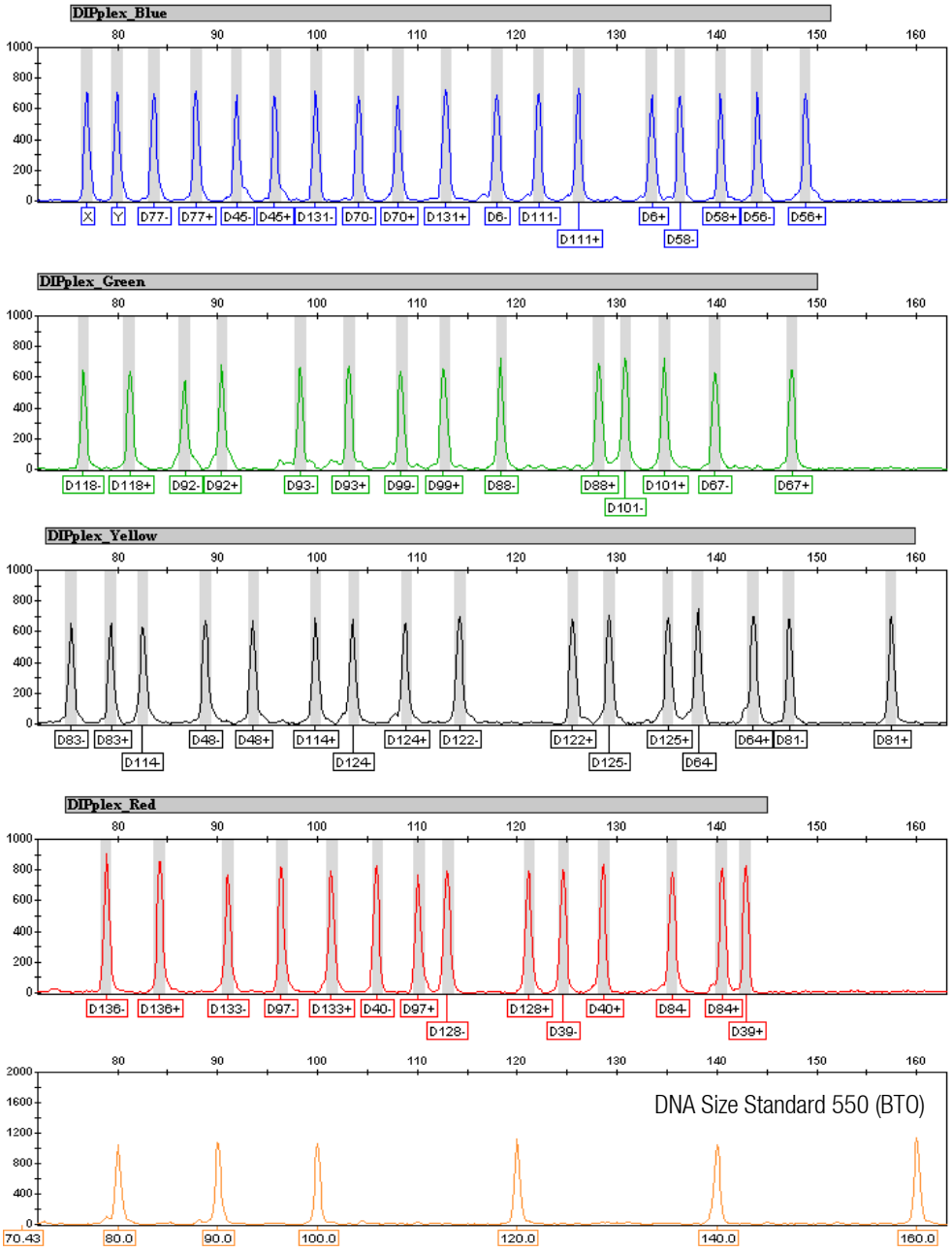

Fig. 7 Electropherogram of the allelic ladder Mentype® **DIPple** .analysed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper™ ID Software and the Mentype® **DIPplex** template file.

Table 4. Fragment lengths of the allelic ladder Mentype® DIPplex analysed on an ABI PRISM® 310/3130 Genetic Analyzer (blue and green panel)

Marker/Blue	-DIP [bp]*	+DIP [bp]*	Marker/Green	-DIP [bp]*	+DIP [bp]*
AM	77 (X)	80 (Y)	**HLD118**	77	81
HLD77	84	88	**HLD92**	87	90
HLD45	92	96	**HLD93**	98	103
HLD131	100	113	**HLD99**	108	113
HLD70	104	108	**HLD88**	118	128
HLD6	118	134	**HLD101**	131	135
HLD111	122	126	**HLD67**	140	147
HLD58	136	140			
HLD56	144	149			

Table 5. Fragment lengths of the allelic ladder Mentype® DIPplex analysed on an ABI PRISM® 310/3130 Genetic Analyzer (yellow and red panel)

Marker/Yellow	-DIP [bp]*	+DIP [bp]*	Marker/Red	-DIP [bp]*	+DIP [bp]*
HLD83	76	80	**HLD136**	79	84
HLD114	83	100	**HLD133**	91	102
HLD48	89	94	**HLD97**	97	110
HLD124	104	109	**HLD40**	106	129
HLD122	115	126	**HLD128**	113	121
HLD125	129	135	**HLD39**	125	143
HLD64	138	144	**HLD84**	135	140
HLD81	147	158			

* rounded to integer

7. Interpretation of results

As mentioned above, post PCR analysis and automatic allele allocation with suitable analysis software ensure a precise and reliable discrimination of alleles.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

Stutter peaks

For STR loci, the appearance of stutter peaks depends on the sequence of the repeat structure and on the number of alleles. In contrast **no** stutter peaks result from DIP structures so that peak assignment of DNA mixtures is much easier. For peak evaluation please see defaults in Genotyper® and GeneMapper™ ID software Template Files.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 peaks). All Biotype® primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68°C for 60 minutes. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their own limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur. Especially at low temperatures loci HLD77, HLD118, HLD92, HLD114, HLD136 und HLD40 are known to display such kind of artefacts. If shoulder or split peaks appear, we recommend injecting the sample again.

Outside the allelic range (>170 bp) amplification artefacts with very low signal intensity may occur.

8. References

Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations. Further report of the DNA Commission of the ISFG regarding the use of short tandem repeat systems. *Int. J. Legal Med. 110: 175-176*.

Gill P, Brenner CH, Buckleton JS, Carracedo A, Krawczak M, Mayr, WR, Morling N, Prinz M, Schneider PM, Weir BS (2006) DNA Commission of the International Society of Forensic Genetics (ISFG): Recommendations on the interpretation of mixtures. *Forensic Sci Int. Jul 13;160 (2-3):90-101.*

Mills RE, Luttig CT, Larkins CE, Beauchamp A, Tsui C, Pittard WS, Devine SE (2006) An initial map of insertion and deletion (INDEL) variation in the human genome. *Genome Res 16 (9):1182-1190, 2006*.

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Weber JL, David D, Heil J, Fan Y, Zhao C, Marth G (2002) Human diallelic insertion/deletion polymorphisms. *Am J Hum Genet 71(4):854-862*.

Notes

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